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Distribution and Macromolecular Binding of Benzo(a)pyrene in SENCAR and BALB/c Mice Following Topical and Oral Administration

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16. ABSTRACT

When benzo[a]pyrene (BaP) is used as the initiator in initiation-promotion assays, the topical route of administration has been shown to produce a greater epidermal tumor incidence than do other routes of administration, particularly the oral route. In addition, different strains of mice exhibit varying degrees of susceptibility to two-stage epidermal tumorigenesis using BaP. The SENCAR strain is known to be far more sensitive to epidermal tumor formation following BaP initiation than are other strains, such as the BALB/c strain. To investigate the possible contribution of distribution and binding to DNA in such route and strain differences, the distribution and macromolecular binding of [^{3}H]BaP was examined in the skin, liver, lung, and stomach of SENCAR and BALB/c mice following topical or oral administration of BaP at time periods ranging from 0.5 to 48 h.

Levels of labeled material in skin were higher, and the binding of BaP to epidermal DNA was greater following topical administration than following oral administration for mice of both strains. The much greater binding of BaP to epidermal DNA following topical administration may account for the much greater epidermal tumor incidence in mice following topical administration of BaP. Following topical administration, BALB/c mice had generally higher levels of labeled material in whole skin than did SENCAR mice, and the binding of BaP to epidermal DNA at 48 h was greater in BALB/c mice than in SENCAR mice following either route of administration. Thus, the known differences between these two strains in susceptibility to epidermal tumor formation when BaP is used as an initiator cannot be explained on the basis of differences in tissue distribution or the amount of binding to epidermal DNA at the time periods examined.

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DISTRIBUTION AND MACROMOLECULAR BINDING OF BENZO[a]PYRENE IN SENCAR AND BALB/c MICE FOLLOWING TOPICAL AND ORAL ADMINISTRATION

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When benzo[a]pyrene (BaP) is used as the initiator in initiation-promotion assays, the topical route of administration has been shown to produce a greater epidermal tumor incidence than do other routes of administration, particularly the oral route. In addition, different strains of mice exhibit varying degrees of susceptibility to two-stage epidermal tumorigenesis using BaP. The SENCAR strain is known to be far more sensitive to epidermal tumor formation following BaP initiation than are other strains, such as the BALB/c strain. To investigate the possible contribution of distribution and binding to DNA in such route and strain differences, the distribution and macromolecular binding of [^{3}H]BaP was examined in the skin, liver, lung, and stomach of SENCAR and BALB/c mice following topical or oral administration of BaP at time periods ranging from 0.5 to 48 h.

Levels of labeled material in skin were higher, and the binding of BaP to epidermal DNA was greater following topical administration than following oral administration for mice of both strains. The much greater binding of BaP to epidermal DNA following topical administration may account for the much greater epidermal tumor incidence in mice following topical administration of BaP. Following topical administration, BALB/c mice had generally higher levels of labeled material in whole skin than did SENCAR mice, and the binding of BaP to epidermal DNA at 48 h was greater in BALB/c mice than in SENCAR mice following either route of administration. Thus, the known differences between these two strains in susceptibility to epidermal tumor formation when BaP is used as an initiator cannot be explained on the basis of differences in tissue distribution or the amount of binding to epidermal DNA at the time periods examined.

INTRODUCTION

The initiation-promotion assay is one of a number of short-term in vivo test systems used for evaluation of the tumorigenic/carcinogenic

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potential of chemicals (Weisburger and Williams, 1981). When polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP) are used as initiators, results obtained using this assay can vary dramatically, depending upon the route of administration or mouse strain used.

Bull et al. (1981) found that both the proportion of SENCAR mice developing papillomas and the number of papillomas per mouse were greater when BaP was administered topically than when BaP was administered sc, ip, or orally in initiation-promotion assays. The greatest differences in tumor incidence were observed between the topical and oral routes. In contrast, these authors found a greater epidermal tumor incidence in SENCAR mice after oral administration of ethyl carbamate than after topical administration of ethyl carbamate. Hennings et al. (1981) found SENCAR mice to be far more susceptible than BALB/c mice to epidermal papilloma or carcinoma formation using 7,12-dimethylbenz[a]anthracene as the initiator. Bull et al. (1983) tested various strains of mice in a combined pulmonary adenoma and initiation-promotion assay in which BaP and other carcinogens were administered ip followed by promotion with 12-O-tetradecanoylphorbol 13-acetate for 20 wk. After 36 wk, SENCAR mice treated with BaP had a greater incidence of pulmonary adenomas and epidermal papillomas than did controls, while BALB/c mice did not exhibit an increased incidence in either tumor type with BaP initiation.

These route and strain differences in epidermal tumor incidence could be a result of differences in various phenomena related to tumor initiation, including differences in the distribution of BaP, in the metabolic activation or detoxification of BaP, or in the binding of metabolites of BaP to cellular macromolecules such as DNA.

In this study, the distribution of [^{3}H]BaP in SENCAR and BALB/c mice after topical or oral administration was determined at time intervals ranging from 0.5 to 48 h after dosing. The organs selected for study included the skin, lung, and stomach, all of which are target organs for BaP tumorigenesis, and the liver, which, though not a target organ, is metabolically active with respect to BaP. In addition, the binding of [^{3}H]BaP to DNA, RNA, and protein of epidermis, liver, lung, and stomach was examined at 6 and 48 h after topical or oral administration in both strains of mice.

MATERIALS AND METHODS

Animals

Male SENCAR and BALB/c mice were obtained from Harlan Sprague-Dawley of Indianapolis, Ind. Mice used in these experiments were 5-8 wk of age and weighed 18-25 g.

Chemicals

[G-^{3}H]Benzo[a]pyrene (25 μCi/mmol) was obtained from Amersham Corporation, Arlington Heights, Ill. Unlabeled benzo[a]pyrene, bovine serum albumin (fraction V), Folin-Ciocalteu phenol reagent, and fungal protease K were obtained from Sigma Chemical Company of St. Louis, Mo. All other chemicals were reagent grade or purer.

Treatment of Animals

In all studies, mice were individually housed in stainless-steel metabolism cages and deprived of food but not water 24 h prior to treatment. An area of 6-7 cm^2 on the back of each mouse was shaved using surgical clippers 36-48 h before dosing. [^{3}H]BaP was administered orally via intubation in an emulsion of 10% GAF Emulphor EL 620 and 90% H_2O or was applied topically dissolved in acetone. These vehicles were the same as those employed by Bull et al. (1981) in the initiation-promotion assays. The specific activities of the dosing solutions were 630 μCi/mmol and 25.2 mCi/mmol for the distribution and binding studies, respectively. Animals were sacrificed via cervical dislocation at 0.5, 1, 6, 12, 24, or 48 h after dosing in the distribution studies, and at 6 or 48 h in the binding studies.

The dose of BaP selected for these experiments was 50 mg/kg. At that dose, 100% of topically treated SENCAR mice develop papillomas, less than 15% of orally treated SENCAR mice develop papillomas, and BALB/c mice given BaP orally show no papilloma formation (R. Bull, 1984, personal communication). BALB/c mice were not tested at this dose level by topical administration, but at 10 mg/kg, only 16% of topically treated BALB/c mice developed papillomas, compared to $>$ 90% incidence in SENCAR mice at 1 mg/kg. Hence, while the 50-mg/kg dose of BaP is quite high, it does represent a dose level at which both strain and route differences in tumorigenic susceptibility to BaP are expected to occur.

[^{3}H]BaP Distribution: Processing and Analysis of Samples

Duplicate 50- to 100-mg samples of skin, liver, lung, and stomach of each animal were taken. One ml of Amersham NCS solubilizer and 0.2 ml of H_2O_2 were added to each sample prior to incubation at 50°C in a Dubnoff shaking incubator for 24 h. Following incubation, 10 ml New England Nuclear Aquasol-2 was added to each sample. The ^{3}H content was determined by liquid scintillation counting with ICN [^{3}H]toluene used as an internal standard.

Strain differences in total ^{3}H activity for a given tissue, route, and time were assessed by use of a two-level nested analysis of variance ($p <$ 0.05).

[^{3}H]BaP Binding: Isolation of Macromolecules and Analysis

At the time of sacrifice, the liver, lungs, and stomach of each mouse were excised, as well as the treated area of the skin from the back. DNA, RNA, and protein were isolated by a combination of the modified techniques of Kirby and Cook (1967) and Marmur (1963).

Skins of animals were randomly assigned to pooled samples consisting of three skins. Epidermal layers were removed by a thermal method (Slaga et al., 1974). Two to four ml of an aqueous solution (6% *p*-aminosalicylate, 1% NaCl, 1% triisopropyl naphthalene sulfonate, and 6% sec-butyl alcohol) were added to the pooled epidermal scrapings prior to homogenization via a Polytron PT-10 at a setting of 6 for 30-45 s. Unpooled liver, lung, and stomach samples were homogenized in the aqueous solution (2 ml solution/100 mg tissue) using glass Potter-Elvehjem tissue grinders.

An equal volume of chloroform/isoamyl alcohol (24:1 v/v) was added to all samples. After centrifugation at 12,000×g and 5°C for 30 min, the aqueous layer of each sample was removed, as well as the protein pellet. The chloroform/isoamyl alcohol extraction was repeated once on the aqueous layer of liver, lung, and stomach samples, and three times on that of the epidermal samples.

To each of the epidermal DNA samples, 0.2 ml protease K solution (131 units/ml in 1% sodium dodecyl sulfate, 1 m*M* EDTA, and 10 m*M* Tris-HCl, pH 8.0) was added. Following incubation for 12 h at 37°C, the samples were extracted twice with chloroform/isoamyl alcohol. DNA was precipitated by the addition of an equal volume of 2-ethoxyethanol for all samples. Following removal of DNA, an equal volume of ethanol was added to the remaining solution, and samples were refrigerated to allow precipitation of RNA.

Nucleic acids were quantitated spectrophotometrically at 260 nm using $E = 251$ and $E = 210$ for DNA and RNA, respectively. The method of Lowry et al. (1951) was used to quantitate protein samples. The ^{3}H content of samples was quantitated by scintillation counting.

Strain and route differences in the binding of BaP equivalents to a given macromolecule for a given tissue and time after dosing were assessed by two-way analysis of variance followed by a Newman-Keuls ranges test ($p < 0.05$).

RESULTS

Distribution of [^{3}H]BaP

When [^{3}H]BaP was administered orally or topically to SENCAR and BALB/c mice at times ranging from 30 min to 48 h, levels of ^{3}H in the skin of mice of both strains were much greater following topical administration of BaP than after oral administration (Fig. 1). Following topical administration, levels of labeled material in the skin were generally

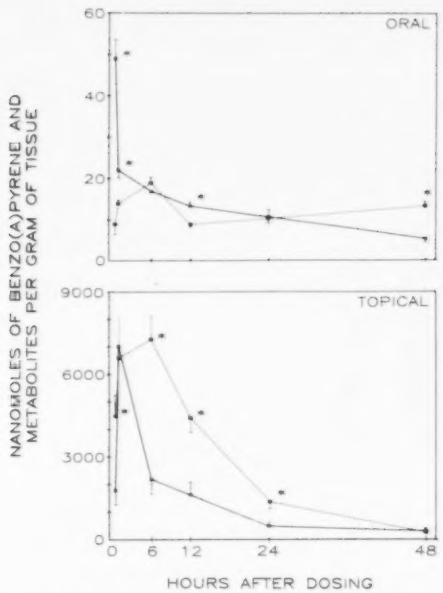

FIGURE 1. Distribution of [^{3}H]benzo[a]pyrene in skin following oral or topical administration to SENCAR and BALB/c mice. [^{3}H]Benzo[a]pyrene (50 mg/kg, 630 μCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR mice (solid line) and BALB/c mice (broken line). Values are expressed as nanomoles of benzo[a]pyrene and metabolites per gram of tissue. Each value represents the mean ± SEM of 4-14 mice. Asterisks denote statistically significant differences between strains at given time points for a single route ($p < 0.05$).

higher in BALB/c mice than in SENCAR mice. Following oral administration of BaP, levels were initially higher in SENCAR mice than in BALB/c mice, but this difference did not persist beyond the 12-h measurement.

Oral administration of [^{3}H]BaP resulted in greater levels of [^{3}H]BaP equivalents in liver, lung, and stomach tissue of both strains than did topical administration (Figs. 2-4). Few differences between strains in the distribution of labeled material in these three organs were noted following oral administration of BaP. After topical administration, levels of labeled material in the liver (Fig. 2) and lung (Fig. 3) of SENCAR mice were greater than those found in the corresponding organs of BALB/c mice. This strain difference was not as pronounced in stomach tissue following topical administration (Fig. 4).

Covalent Binding of [^{3}H]BaP to DNA, RNA, and Protein

In order to better evaluate the actual binding of [^{3}H]BaP to macromolecules, binding to DNA, RNA, and protein was determined at 6 and 48 h after administration of BaP to both strains by both routes. Binding of BaP to epidermal DNA, RNA, and protein in both strains was much

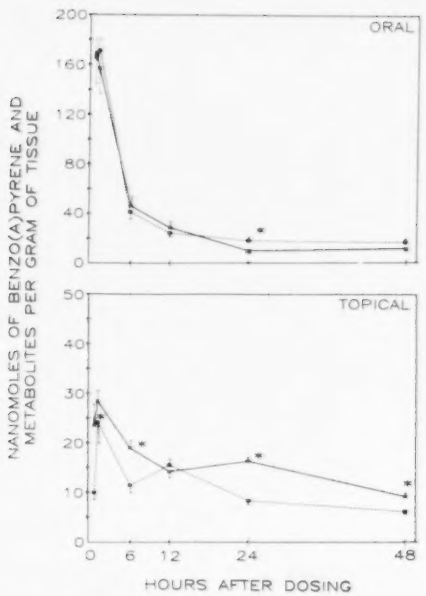

FIGURE 2. Distribution of [^{3}H]benzo[a]pyrene in liver following oral or topical administration to SENCAR and BALB/c mice. [^{3}H]Benzo[a]pyrene (50 mg/kg, 630 μCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR mice (solid line) and BALB/c mice (broken line). Values are expressed as nanomoles of benzo[a]pyrene and metabolites per gram of tissue. Each value represents the mean ± SEM of 4-14 mice. Asterisks denote statistically significant differences between strains at given time points for a single route ($p < 0.05$).

greater following topical administration of BaP than after oral administration at both time periods examined (Fig. 5). For epidermal DNA, the difference in the level of binding between the 2 routes was at least 20-fold at 48 h after administration. Strain differences in the binding of BaP to epidermal DNA were evident at both time periods: at 6 h, binding to epidermal DNA was greater in SENCAR mice after either route of administration, while at 48 h, binding to epidermal DNA was greater in BALB/c mice after either route of administration. However, only those strain differences between groups of animals that were treated topically were statistically significant.

Binding of BaP to DNA, RNA, and protein was greater following oral administration than after topical administration in the liver and stomach tissue at both time periods (Figs. 6 and 8), and in the lung at 6 h (Fig. 7). At 48 h, binding of BaP to DNA, RNA, and protein in lung was greater after topical administration of BaP than after oral administration. Strain differences in the macromolecular binding of BaP in liver, lung, and stomach were not consistent except for liver at 48 h, where binding to DNA, RNA, and protein of orally treated SENCAR mice was greater than that of orally treated BALB/c mice.

DISCUSSION

Brookes and Lawley (1964) found a correlation between the tumorigenicity of several PAHs, including BaP, and the ability of these PAHs to bind covalently to DNA of the skin of Swiss mice in vivo. Buty et al. (1976) in in vitro studies employing epidermal homogenates of CD-1 mice also found a correlation between the tumorigenicity of several PAHs and their ability to bind covalently to DNA. More recently, Ashurst et al. (1983) found a similar dose dependency between the number of papillomas per mouse and either the total amount of [^{3}H]BaP bound to epidermal DNA or the amount of the *7R-anti*-BPDE/*trans*-deoxyguanosine adduct formed in SENCAR mice following topical administration of BaP.

Few, if any, correlations have been found between the ability of a given PAH to bind to epidermal DNA in different mouse strains and the ability of a PAH to elicit tumor formation in different strains of mice. Phillips et al. (1978) found no correlation between the susceptibility of Swiss, C57Bl, and DBA/2 mice to epidermal tumorigenesis induced by BaP, 7,12-dimethylbenz[a]anthracene, or 3-methylcholanthrene and the degree of binding of these PAHs to epidermal DNA in these strains.

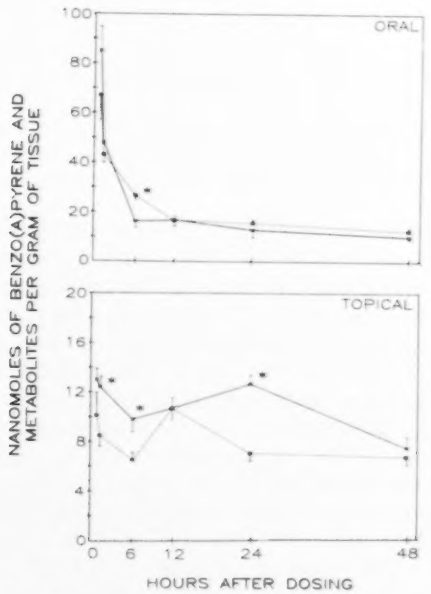

FIGURE 3. Distribution of [^{3}H]benzo[a]pyrene in lung following oral or topical administration to SENCAR and BALB/c mice. [^{3}H]Benzo[a]pyrene (50 mg/kg, 630 μCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR mice (solid line) and BALB/c mice (broken line). Values are expressed as nanomoles of benzo[a]pyrene and metabolites per gram of tissue. Each value represents the mean ± SEM of 4-14 mice. Asterisks denote statistically significant differences between strains at given time points for a single route ($p < 0.05$).

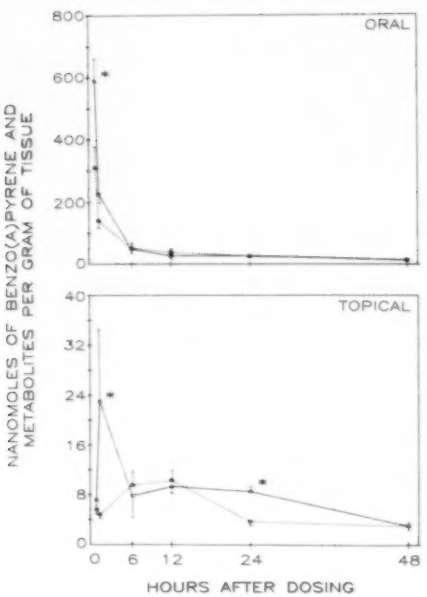

FIGURE 4. Distribution of [^{3}H]benzo[a]pyrene in stomach tissue following oral or topical administration to SENCAR and BALB/c mice. [^{3}H]Benzo[a]pyrene (50 mg/kg, 630 μCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR mice (solid line) and BALB/c mice (broken line). Values are expressed as nanomoles of benzo[a]pyrene and metabolites per gram of tissue. Each value represents the mean ± SEM of 4-14 mice. Asterisks denote statistically significant differences between strains at given time points for a single route ($p < 0.05$).

Pelkonen et al. (1979) and Legraverend et al. (1980) were also unable to establish such correlations in in vitro binding studies using different strains of mice. Ashurst and Cohen (1981) examined DNA adduct formation in Swiss, C57Bl/6, and DBA/2 mice following topical administration of BaP and found similar proportions of various DNA adducts in all three strains. Abbott (1983) found that TO, C57Bl, and DBA/2 mice displayed different sensitivities to tumor formation induced by 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one, although no major differences in total binding of this carcinogen to DNA or in the DNA adduct profiles were found. However, prior to the present study, the in vivo binding of BaP to epidermal DNA in SENCAR and BALB/c mice had not been directly compared, nor had the effects of different routes of administration on the binding of BaP to macromolecules including epidermal DNA been examined in these strains.

A number of investigators have examined the role of promotion as the basis of strain differences in susceptibility to two-stage carcinogenesis. When Sisskin et al. (1982) examined the responses to C57Bl, DBA, BALB/c, and CD-1 mice to hyperplasia induced by TPA, they found no

correlation between sensitivity to tumor promotion and the degree of hyperplasia induced by a single dose of TPA. They did find a better relationship between sensitivity to tumor promotion and TPA-induced hyperplasia after multiple exposures. In this study, the DBA strain appeared to be an outlier, showing as great a hyperplastic response as the other mouse strains, despite being resistant to two-stage carcinogenesis initiated by PAH such as DMBA (Kinoshita and Gelboin, 1972).

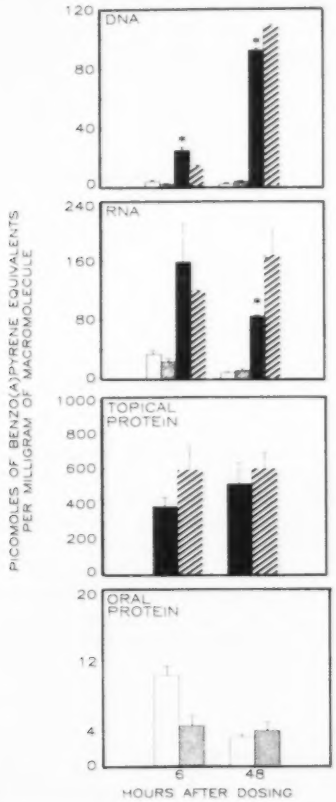

FIGURE 5. Effect of route and strain on the binding of benzo[a]pyrene equivalents to epidermal DNA, RNA, and protein. [^{3}H]Benzo[a]pyrene (50 mg/kg, 25.2 mCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR and BALB/c mice (n = 12). Values are expressed as picomoles of benzo[a]pyrene equivalents per milligram of macromolecule. Each value depicted represents the mean ± SEM of three to four pooled epidermal samples. Open bars represent values of orally treated SENCAR mice; single-hatched bars represent values of orally treated BALB/c mice; solid bars represent values of topically treated SENCAR mice; double-hatched bars represent values of topically treated BALB/c mice. All route differences for a single strain at a given time point are statistically significant. Asterisks denote statistically significant differences between strains for a given route and time ($p < 0.05$).

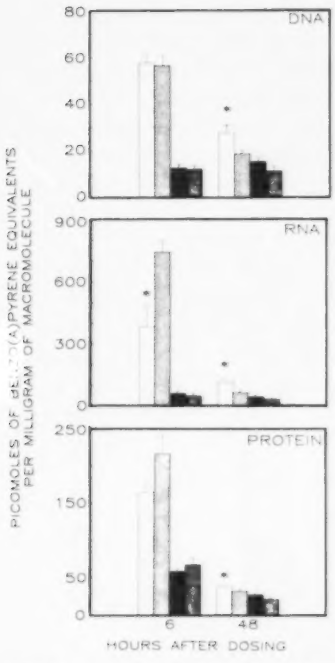

FIGURE 6. Effect of route and strain on the binding of benzo[a]pyrene equivalents to liver DNA, RNA, and protein. [^{3}H]Benzo[a]pyrene (50 mg/kg, 25.2 mCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR and BALB/c mice. Values are expressed as picomoles of benzo[a]pyrene equivalents per milligram of macromolecule. Each value depicted represents the mean ± SEM of 5-7 mice. Open bars represent values of orally treated SENCAR mice; single-hatched bars represent values of orally treated BALB/c mice; solid bars represent values of topically treated SENCAR mice; double-hatched bars represent values of topically treated BALB/c mice. All route differences for a single strain at a given time point are statistically significant. Asterisks denote statistically significant differences between strains for a given route and time ($p < 0.05$).

DiGiovanni et al. (1984) tested SENCAR, C57Bl/6, and DBA/2 mice for sensitivity to two-stage carcinogenesis using DMBA or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) as initiators followed by promotion with TPA. SENCAR mice were shown to be most sensitive when DMBA was used as the initiator. When the direct-acting carcinogen MNNG was used as the initiator, SENCAR and DBA/2 mice were found to be equally sensitive. C57Bl/6 mice were found to be refractory to tumor promotion when either DMBA or MNNG was used as the initiator. Reiners et al. (1984) also found that the C57Bl/6 strain was resistant to promotion with TPA using BaP or DMBA as initiators, but that this strain was more sensitive to promotion with benzoyl peroxide and very sensitive to tumor

formation in complete carcinogenesis protocols using either BaP or DMBA.

On the basis of the studies just cited, it seems that some of the reported differences among various mouse strains to tumor formation induced by PAH in initiation-promotion assays can be explained by differences in the promotion stage, while some strain differences cannot. The C57Bl/6 strain does seem to be refractory to promotion by phorbol esters (DiGiovanni et al., 1984; Reiners et al., 1984). The DBA/2 and SENCAR strains are quite sensitive to TPA promotion, although the DBA/2 strain is refractory to initiation by PAH (DiGiovanni et al., 1984; Kinoshita and Gelboin, 1972). The BALB/c strain has not been clearly

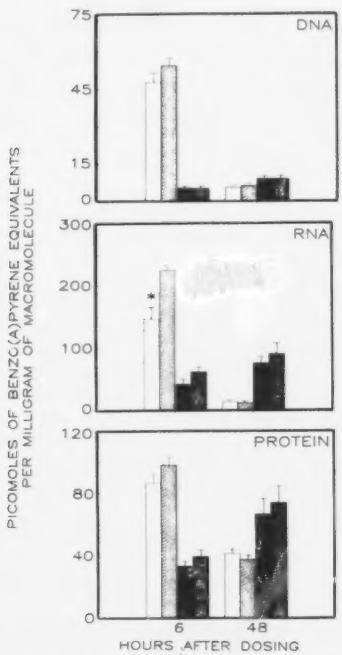

FIGURE 7. Effect of route and strain on the binding of benzo[a]pyrene equivalents to lung DNA, RNA, and protein. [^{3}H]Benzo[a]pyrene (50 mg/kg, 25.2 mCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR and BALB/c mice. Values are expressed as picomoles of benzo[a]pyrene equivalents per milligram of macromolecule. Each value depicted represents the mean ± SEM of five to seven mice. Open bars represent values of orally treated SENCAR mice; single-hatched bars represent values of orally treated BALB/c mice; solid bars represent values of topically treated SENCAR mice; double-hatched bars represent values of topically treated BALB/c mice. All route differences for a single strain at a given time point are statistically significant. Asterisks denote a statistically significant difference between strains for a given route and time ($p < 0.05$).

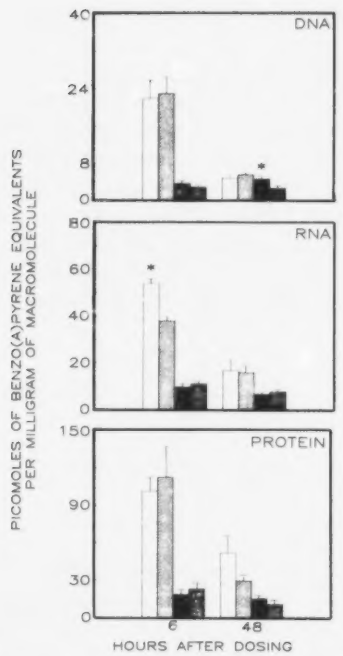

FIGURE 8. Effect of route and strain on the binding of benzo[a]pyrene equivalents to stomach DNA, RNA, and protein. [^{3}H]Benzo[a]pyrene (50 mg/kg, 25.2 mCi/mmol) was administered orally in an emulsion or topically in acetone to male SENCAR and BALB/c mice. Values are expressed as picomoles of benzo[a]pyrene equivalents per milligram of macromolecule. Each value depicted represents the mean ± SEM of five to seven mice. Open bars represent values of orally treated SENCAR mice; single-hatched bars represent values of orally treated BALB/c mice; solid bars represent values of topically treated SENCAR mice; double-hatched bars represent values of topically treated BALB/c mice. All route differences for a single strain at a given time point are statistically significant. Asterisks denote statistically significant differences between strains for a given route and time ($p < 0.05$).

shown to be resistant to promotion with phorbol esters. Hennings et al. (1981) tested both SENCAR and BALB/c mice in initiation-promotion assays using both DMBA and MNNG as initiators. Although the average number of papillomas per mouse was greater in SENCAR mice than in BALB/c mice using MNNG as the initiator, the carcinoma incidence in adult animals was similar for both strains at both doses tested. Also, newborn SENCAR and BALB/c mice were both found to be generally insensitive to tumor induction following administration of MNNG, while only newborn SENCAR mice were found to be sensitive to tumor induction using DMBA as an initiator.

The differences found between the topical and oral routes of administration in each strain do relate to the differences in the tumorigenicity of these two routes found by Bull et al. (1981). Levels of labeled material in whole skin and the binding of BaP to epidermal DNA at 48 h were found to be much greater for either strain after topical administration than after oral administration. It is quite possible that the greater binding of BaP to epidermal DNA following topical administration is sufficient to account for the greater tumor formation in mice that occurs after topical administration of BaP than after oral administration of BaP, and that this greater binding to epidermal DNA is due to the greater availability of BaP for binding following topical administration.

In the distribution studies, BALB/c mice had generally higher levels of labeled material in skin than did SENCAR mice following topical administration of BaP. Although binding of BaP to epidermal DNA was greater in SENCAR mice than in BALB/c mice at 6 h after administration, at 48 h the binding of BaP to epidermal DNA was greater in BALB/c mice. Such differences do not indicate then why SENCAR mice are more susceptible to tumor formation in initiation-promotion assays using BaP. It is possible that in vivo epidermal DNA adduct profiles in the two strains vary. In addition, strain differences in the total covalent binding of BaP to epidermal DNA or in the amount of the *7R-anti*-BPDE/*trans*-deoxyguanosine adduct formed may be evident at much longer time intervals after administration of BaP than were used in this study. Such possibilities should be examined before differences in the binding of BaP to epidermal DNA are ruled out as a potential source of the known differences in the susceptibility of SENCAR and BALB/c mice to tumor induction using BaP.

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